AMP cause induction of the enzyme, two separate regulatory systems may be involved, possibly dependent on separate structural genes.

Since we detected little or no adenyl cyclase or cyclic AMP in the HTC cells (results obtained were at the limits of sensitivity of the methods used), it appears likely that this line of mammalian cells is deficient in these components. The induction of hepatic TAT by glucagon in vivo is probably mediated by cyclic AMP; thus, the lack of response to glucagon in HTC cells might be attributable to a deficiency of adenyl cyclase, lack of sensitivity of the cells to the cyclic nucleotide, or both. In any event, our data suggest that induction of TAT by glucocorticoids in HTC cells does not involve the adenyl cyclase-cyclic AMP system.

D. GRANNER*
Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland 20014
L. R. CHASE
G. D. AURBACH
Section on Mineral Metabolism, Metabolic Diseases Branch, National Institute of Arthritis and Metabolic Diseases, Bethesda
G. M. TOMKINS
Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, Bethesda

References and Notes

1. F. T. Kenney, *J. Biol. Chem.* **237**, 3495 (1962).
2. O. Barnabei and F. Sereni, *Boll. Soc. Ital. Biol. Sper.* **36**, 1656 (1960); L. Goldstein, E. J. Stella, W. E. Knox, *J. Biol. Chem.* **237**, 1723 (1962); C. B. Hager and F. T. Kenney, *ibid.* **243**, 3301 (1968).
3. W. D. Wicks, *J. Biol. Chem.* **243**, 900 (1968).
4. D. D. Holten and F. T. Kenney, *ibid.* **242**, 4372 (1967).
5. W. D. Wicks, *Science* **160**, 997 (1968).
6. O. Greengard and H. K. Dewey, *J. Biol. Chem.* **242**, 2986 (1967).
7. E. W. Sutherland, G. A. Robison, R. W. Butcher, *Circulation* **37**, 279 (1968).
8. C. M. Szego and J. S. Davis, *Proc. Nat. Acad. Sci. U.S.* **58**, 1711 (1967).
9. G. W. Sharp, M. A. Kirchberger, D. G. Martin, A. Leaf, *J. Clin. Invest.* **47**, 89a (1968).
10. E. B. Thompson, G. M. Tomkins, J. M. Curran, *Proc. Nat. Acad. Sci. U.S.* **56**, 296 (1966).
11. D. K. Granner, S. Hayashi, E. B. Thompson, G. M. Tomkins, *J. Mol. Biol.* **35**, 291 (1968).
12. L. R. Chase and G. D. Aurbach, *Science* **159**, 545 (1968).
13. T. I. Diamondstone, *Anal. Biochem.* **16**, 395 (1966).
14. The findings that HTC cells contain active cyclic nucleotide phosphodiesterase suggested to us that the substrate (cyclic AMP) for this enzyme might be present in these cells. Whole homogenate from HTC cells hydrolyzed 75 percent of a $10^{-6}M$ solution of cyclic AMP to 5′-AMP in 10 minutes when a quantity of tissue was tested equivalent to that used for the assay of adenyl cyclase. Disappearance of tritiated cyclic AMP was measured after removal of 5′-AMP from solution by zinc sulfate and barium hydroxide. The concentration of theophylline used in the assay for adenyl cyclase was sufficient to completely block any phosphodiesterase contamination of the cell membrane fraction.
15. L. R. Chase and G. D. Aurbach, *Proc. Nat. Acad. Sci. U.S.* **58**, 518 (1967); G. D. Aurbach and B. A. Houston, *J. Biol. Chem.*, in press.
16. I. Pastan, *Biochem. Biophys. Res. Commun.* **25**, 14 (1966).
17. We thank Mrs. S. Fedak and Mr. C. Woodard for expert technical assistance.
* Present address: Department of Internal Medicine, University of Wisconsin Medical Center, Madison.

5 September 1968

Pulmonary and Circulatory Adjustments Determining the Limits of Depths in Breathhold Diving

Abstract. *Data on pulmonary gas exchange were collected in breathhold dives to 90 feet in a tank and in open-sea breathhold dives to depths of 217.5 and 225 feet. Thoracic blood volume displacements were measured at depths of 25, 50, 90, and 130 feet, by use of the impedance plethysmograph. The open-sea dives were carried out with an average speed of descent of 3.95 feet per second and an average rate of ascent of 3.50 feet per second. End-dive alveolar oxygen tensions did not fall below 36 millimeters of mercury, while alveolar carbon dioxide tension did not rise above 40 millimeters of mercury except in one case. These findings indicate that for diver Croft, who has unusual lung capacity, neither hypoxia nor hypercapnia determined the depth limits under those conditions. At depths of 90 and 130 feet blood was forced into the thorax, amounting to 1047 and 850 milliliters respectively.*

Recent exploits in breathhold diving have shown that man has the capacity to dive to depths in excess of 200 feet (60 m) (*1*). This raises questions about the validity of the generally held assumption that the depth threshold is determined by a point at which total lung volume (*2*) (the amount of gas contained in the lungs at the end of maximal inspiration) is compressed to the residual volume (volume of gas in lungs at end of maximal expiration). Both recent record-holders in breathhold diving, Robert Croft (217.5 feet) and Jacques Mayol (231 feet), went to considerably greater depths than could be predicted on the basis of their total lung volume/residual volume ratios. Additional factors must therefore be considered. It has been suggested that at greater depths blood is forced into the thorax, replacing air and resulting in a decrease of residual volume and thereby extending the depth limit (*3*, *4*).

This report presents the results of the first measurements of thoracic blood volume made in breathhold dives, demonstrating a shift in blood volume into the thorax at great depths. Moreover, end-dive alveolar gas tensions were obtained in dives up to 225 feet in the open sea, considerably extending the depth range of information in pulmonary gas exchange in breathhold diving.

Studies were carried out on Robert Croft, a U.S. Navy diver, to depths of 90 feet at the Escape Training Tank, Naval Submarine Base, Groton, Connecticut, and extended to open-sea dives off Fort Lauderdale, Florida, culminating in Croft's world-record dives to 217.5 feet and recently to 240 feet. The 240-foot dive was covered by three underwater photographers, who observed a pronounced caving in of the thorax and compression of the abdomen at depth, which may be seen in the cover pictures. They also noticed skinfolds flapping around the chest.

The vital statistics of R. Croft (Table 1) show his unusually large vital capacity and small residual volume. Based on the ratio of total lung volume (total lung capacity) to residual volume his depth threshold would be 197 feet, which is considerably deeper than that of the average person (80 to 100 feet). Moreover, R. Croft is able to exert much larger expiratory pressures and somewhat larger inspiratory pressures than those found in normal healthy subjects. (The expiratory and inspiratory pressures were measured at various lung volumes varying from residual volume to total lung capacity).

Before the dives in the open sea, the subject sat on a platform up to his waist in water. He lowered himself into the water up to the neck and held on to the raft in front of him, which supported two investigators who collected alveolar and expired gas samples. The breathing valve was attached to the raft in a low position. The subject breathed through a tube 1¼ inches (3.2 cm) in diameter into rubber bags. A two-way stop cock allowed switching from one bag to another at the end of collection periods. The filled bags were clamped and stored until the end of the experiment. The

volume of the bag was determined with a dry-gas meter. Gas analysis was carried out with an infrared CO_2 meter (Godard) and a Beckman model 777 oxygen meter.

At the end of the dive the subject made his first exhalation into a two-bag system previously used in diving studies (*5*) or a one-bag system with a Hans Rudolph low dead space (18 ml) valve. Following the collection of the end-dive sample, the subject continued to breathe into the rubber bags, until the end of the experiment. Expired air was collected at ½- or 1-minute intervals during the first 5 minutes then in 2- to 3-minute intervals.

A four-electrode, 120 hertz impedance plethysmograph was used to measure changes in the subject's thoracic resistance related to displaced blood volume during breathhold dives. In the four-electrode system, current (0.12 ma at 120 mv) is introduced to an outer set of electrodes (I_1, I_2) and variation in conduction of the current as a function of thoracic gas or blood volume is detected between inner electrodes (E_1, E_2) (*6*). Two pairs of lead strips (1 cm wide by 20 cm long) were fastened horizontally to a sponge pad (30 by 20 by 1 cm). The two strips of a pair were separated by 1 cm, and one pair was spaced 30 cm from the other. The pad was placed on the back of the subject with the inner strip (E_1) of the upper pair at the level of C_7 (7th cervical vertebra), and the inner strip (E_2) of the lower pair at T_{12} (12th thoracic vertebra). Velcro straps, fastened to the upper and lower edges of the sponge pad, crossed over the front of the chest and held the electrode pad in place under the diver's wet-suit jacket. The lead strips were soldered to insulated wires which terminated in a four-pin connector brought from beneath the wet-suit jacket for connection with a shielded cable of approximately 300 feet in length which was connected to the impedance plethysmograph.

After application of electrodes to the subject, the plethysmograph unit was balanced by means of a variable potentiometer. This null balance represented the resistance of the subject's thorax between detecting electrodes (E_1-E_2).

Under the experimental conditions of this study, movement artifacts were not encountered as a major difficulty.

Calculations of blood volume were obtained in the following manner:

$V_o = \rho(L^2/R_o)$

$V = (150/R_o) \times [(L^2 \times \Delta R)/R_o]$

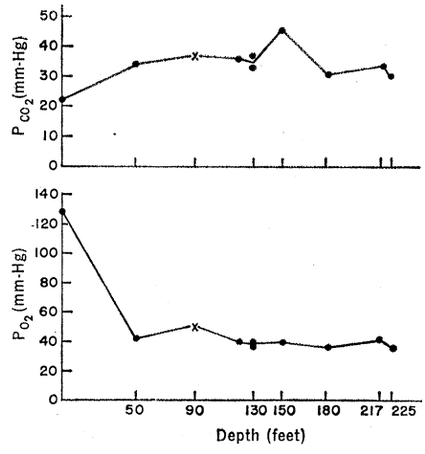

Fig. 1. End-dive alveolar gas tension obtained after rapid ascent at an average rate of 1.2 m/sec from various depths. Control values (leftmost): alveolar gas tensions after rapid exhalation following maximal inhalation. X, tank dive; circle, open-sea dive.

ρ = resistivity of blood at 37°C, 150 (ohm/cm)

L^2 = distance (cm) between detecting electrodes (E_1-E_2)

R_o = resistance (ohms) of the thorax when the plethysmograph is balanced

Output of the plethysmograph was recorded on a Sanborn polygraph series 150. Expiration and increased blood volume are associated with decreased thoracic resistance. No attempt was made to interpret superimposed pulsatile changes related to the cardiac cycle in this study.

Evidence for the validity of the impedance method to detect blood volume changes in the lungs has been described (*6, 7*). Measurements of blood, pulse rate, and blood flow by using electromagnetic flow meters and direct bleed-out methods simultaneously with impedance plethysmograph studies in dogs agreed within 4 percent ± 5 percent (*6*).

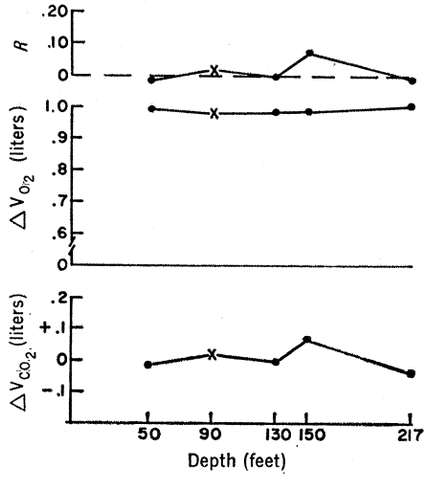

Fig. 2. Gas exchange in breathhold dives to different depths. Oxygen consumed and CO_2 accumulated during the individual dives has been determined by subtracting the end-dive oxygen and CO_2 content of the lungs from control values measures prior to the dive. X, tank dive; circle, open-sea dive; *R*, respiratory exchange ratio.

After a typical period of hyperventilation prior to a dive in the open sea, alveolar CO_2 tension decreased to 22.1 mm-Hg while alveolar O_2 tension rose to 132 mm-Hg (Fig. 1). At the end of breathhold dives to various depths both alveolar CO_2 tension and alveolar O_2 tension remained at approximately the same level, averaging 35 mm-Hg P_{CO_2} and 40 mm-Hg P_{O_2} respectively (Fig. 2). The end-dive alveolar P_{O_2} values obtained for Croft are considerably higher than those for other trained divers after dives to 90 feet, which frequently fell below 30 mm-Hg. The constancy of the end-dive alveolar gas tensions is astonishing in view of the different demands on energy expenditures made during dives to different depths.

Since Croft exhaled about the same amount of air at the end of the dives, both O_2 and CO_2 content of the lungs remained approximately the same, averaging 230 ml of O_2 and 190 ml of CO_2. This oxygen content is considerably higher than the 100 ml left at the breaking point of breathholding in dives previously found in Croft (*8*).

Oxygen used and carbon dioxide accumulated during individual breathhold dives was calculated from the differences between the gas content of the lungs before and after the dives and is plotted in Fig. 2. The lung gas exchange values exhibit the same constancy for different dives as the alveolar gas tensions at the end of the dives. About 1000 ml of O_2 are used while practically no CO_2 is accumulated, resulting in a respiratory exchange ratio near zero.

The excess oxygen, calculated by subtracting control values from those determined during the dives and during the recovery period, provides an estimate of the oxygen requirement for the dive.

Data obtained on R. Croft during breathhold dives with maximal ascent rates (1.07 to 1.4 m/sec) to 50, 90, 130, and 145 feet are shown in Fig. 3. The literature contains only values on breathhold dives to shallower depths and at a slower ascent rate (0.6 to 0.67 m/sec) by Craig (*9*) and Yokoyama (*10*). These data are included for com-

Table 1. Vital statistics of diver Robert Croft.

Item	Measure	Predicted normal (and range)
Age	33 years	
Height	69 inches	
Weight	175 lb (79 kg)	
Vital capacity	7.8 liters (BTPS)	4.9 (3.8 to 6.1) liters (*17*)
Residual volume	1.3 liters (BTPS)	2.0 (1.1 to 2.9) liters (*18*)
Total lung volume*	9.1 liters (BTPS)	6.9 (5.4 to 8.4) liters (*17, 18*)
Ratio: total lung volume/ residual volume	6.96	3.17 (2.43 to 4.59) (*18*)
Compression ratio and depth limit	6.96 = 197 feet depth	3.17 = 72 feet depth
Max. expiratory pressure	290 mm-Hg at 6.4 liters inhalation	(60 to 100 mm-Hg) (*18*)
Max. inspiratory pressure	117 mm-Hg at 3.2 liters inhalation	(60 to 100 mm-Hg) (*18*)

* Total lung capacity.

Table 2. Effect of compression at various depths on thoracic conductive volume.

Depth	Thoracic resistance* (ohms)	Thoracic conductive volume † (liters)	ΔSurface-depth thoracic conductive volume (liters)	Calculated conductive volume changes due to gas volume changes (liters)‡	Conductive thoracic volume changes due to blood volume shifts (liters)
			Fresh water		
Surface	39.3	3.950			
25 feet	34.5	4.452	0.502	0.250	+ 0.252
50 feet	33.1	4.640	.690	.350	+ .340
90 feet	28.2	5.447	1.497	.450	+ 1.047
			Seawater		
Surface	18.0	8.500			
130 feet	13.0	11.850	2.350	1.500	0.850

* Resistance (ohms) between electrodes E_1-E_2 on thorax. † $Vo = (150 \times L2)/Ro$ (L^2 = distance between detecting electrodes). ‡ $\Delta V = (\Delta R \times Vo)/Ro$.

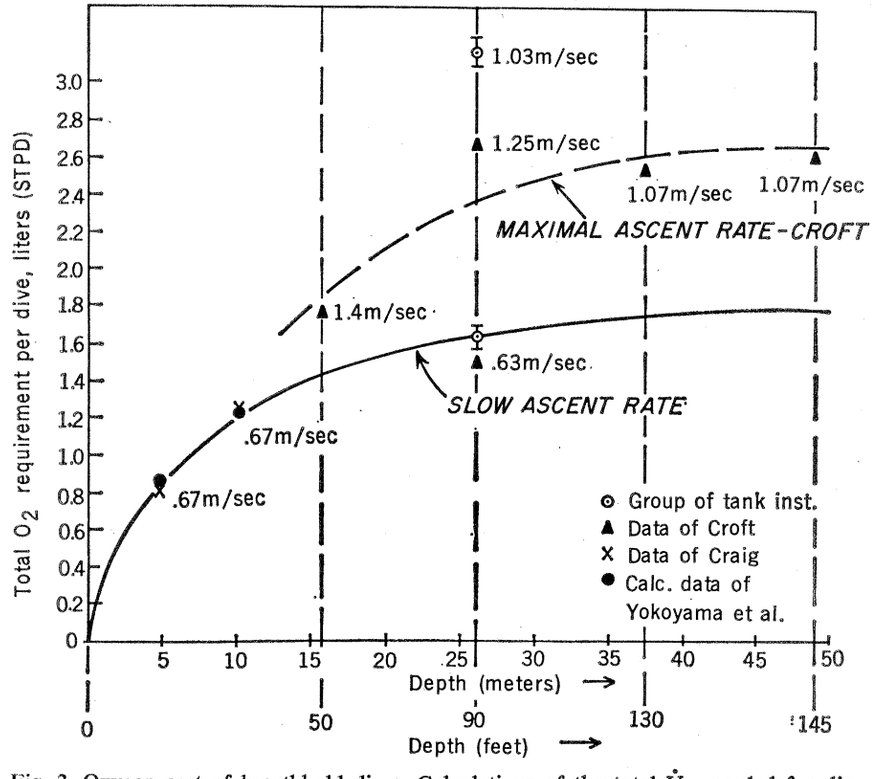

Fig. 3. Oxygen cost of breathhold dives. Calculations of the total $\dot{V}_{O_2}$ needed for dives to different depths were made by subtracting the oxygen consumption measured during the control periods prior to the dive from the oxygen consumed during the dive and during the recovery period after the dive. STPD, standard pressure dry.

parison in Fig. 4 together with other values collected by us in previous studies on R. Croft, and another group of eight divers in breathhold dives to 90 feet using slow and fast ascent rates. It is readily apparent that the oxygen requirements do not increase linearly with depth but instead level off at greater depths for both slow and maximal ascent rates, which is in line with observations of Craig at shallower depths (*9*). The oxygen requirements for dives to 50 feet and above exceed the 1.2 liter capacity of the oxygen stored in the lungs of R. Croft. Therefore, additional oxygen has to be taken from the blood, leading to an increasing unsaturation of the arterial blood and a decreasing rate of transfer of O_2 from the alveoli to the blood. This is one of the factors which might explain the observations that the oxygen requirements in dives to increasing depths increase curvilinearly rather than linearly. It appears that maximum values of oxygen cost in single deep dives lie around 3 liters of oxygen. The average oxygen cost of Croft's dives to 90, 130, and 145 feet was 2.7 liters. At an average diving time of 86 seconds, the oxygen consumption per minute in these single dives amounted to 1.9 liter/min, which is somewhat lower than the reported maximal oxygen consumption ($\dot{V}_{O_2}$) of 3.75 liter/min during exercise (*11*). These findings suggest that circulatory adjustments to diving, resulting in a reduction of O_2 consumption in diving animals, are also in operation in man, which is supported by our recent (unpublished) findings of a marked reduction in cardiac output.

The electrical resistance measurements of the human thorax as determined by impedance plethysmography represents a balance between gas and blood and other tissue volumes within the area defined by the detecting electrode positions (E_1-E_2). Decrease in resistance is associated with decreased gas volume and increased blood volume, and vice versa. Inasmuch as the segmental thoracic resistance relates to the ratio of blood and gas in the thorax, it is correct to speak of a thoracic conductive volume. To arrive at blood volume one has to subtract the changes in gas volumes from the calculated thoracic conductive volume. To accomplish this it is necessary to determine the thoracic resistance at different ratios of gas volume to blood volume in a subject.

Such a calibration was performed with diver Croft in fresh water, sea-

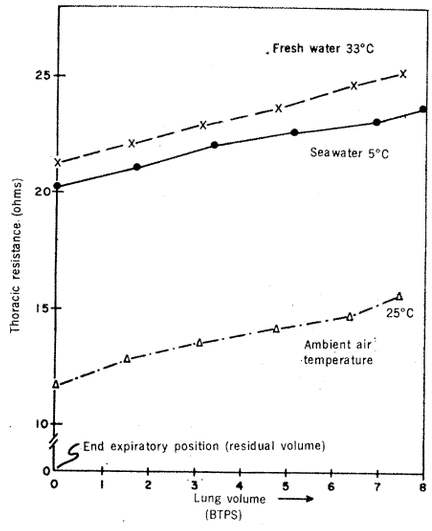

Fig. 4. Thoracic resistance rises nearly linearly with increasing gas volumes measured in seawater, fresh water, and ambient air at different temperatures. The basic thoracic resistance measured at residual volume varies with the temperature of the medium.

water, and air, and thoracic resistance was determined after complete exhalation and inhalation of 1.5, 3.0, 4.5, 6.0, and 7.0 liters of air from a spirometer at ambient temperatures; these values were later converted to BTPS (body temperature pressure saturated). Resistance changed in all three conditions in a linear manner (Fig. 4), although the basic resistance at end expiratory level was different, owing to the different temperature conditions. By using these calibration curves it was possible to calculate the resistance changes produced by compression of gas and by transfer of gas. The latter had been determined for depths up to 90 feet. For 130 feet of seawater the compression ratio was known, but the gas transfer had to be approximated.

Table 2 lists the thoracic resistance changes recorded at various depths in fresh water and seawater. Based on the thoracic resistance changes the thoracic conductive volumes were calculated by using the formula shown in a footnote to Table 2. The thoracic conductive volume changes due to compression of gas and gas transfer from the lungs into the blood shown in column 5 were subtracted from the total thoracic conductive volume changes, leaving the thoracic conductive volume changes due to blood shifts, shown in column 6.

At 90 feet (fresh water) in the Escape Training Tank, 1047 ml of blood were shifted into the thorax, and 850 ml of blood at the 130-foot depth in seawater. These blood volume shifts are in the order of magnitude one would expect from other considerations. Jacques Mayol, who set the most recent world record in breathhold diving, reaching 231 feet, was kind enough to let us have the results of his pulmonary function tests carried out at Broward General Hospital in Fort Lauderdale, Florida, under the direction of Dr. W. S. Williams. His vital capacity was 5.281 liters and his residual volume was 1.883 liters. Adding 60 ml for the sinus dead space, we calculated a total lung capacity (total lung volume) of 7.224 liters. The ratio of total lung capacity to residual volume is 3.717 and corresponds to a depth threshold of 90 feet. In order for Mayol to dive to 231 feet a blood shift of 980 ml into the thorax is required with a corresponding replacement of air and reduction of his residual volume to approximately one-half. Studies of respiratory mechanics done during submersion and in air lend further support to the reported blood shifts into the thorax during diving. The intrathoracic pressures remained, during submersion, at the same level as during control conditions (sitting, in air), while the pressures of the extrathoracic vascular system showed an absolute increase (*3*).

Indirect evidence of intrathoracic blood pooling in breathhold diving may be deduced from the observations of a "diving diuresis" commensurate with dehydration of red cells in tank instructors carrying out breathhold dives to 90 feet (*12*) and "water diuresis" of the swimmer (*13*). This suggests the operation of the Gauer-Henry effect, according to which any measure leading to an expansion of intrathoracic blood volume is accompanied by a diuresis (*14*). This volume regulation is controlled by stretch receptors in the left atrium, whose activity decreases with increasing thoracic blood volume, resulting in a reduction of anti-diuretic hormone secretion and associated diuresis (*14*). In contrast to man, diving animals (seals) exhibit a cessation of urinary flow during dives (*15*). There is evidence for the existence of a different mechanism. It has been demonstrated that the response of the seal to an increase in plasma volume is a solute diuresis with hypertonic urine, while man reacts to the same stimulus with a water diuresis (*16*).

Note added in proof: While this report was in preparation, Craig (*19*) reported evidence suggesting a blood shift of 600 ml in dives to 4.75 m following maximal exhalation. He found no significant difference between esophageal and ambient pressure at this depth, indicating that the subject's residual volume of 2.0 liters must have been compressed to 1.4 liters, owing to a corresponding blood shift.

KARL E. SCHAEFER
ROBERT D. ALLISON
JAMES H. DOUGHERTY, JR.
CHARLES R. CAREY, ROGER WALKER
FRANK YOST, DONALD PARKER

Submarine Medical Research Laboratory, Groton, Connecticut, Scott and White Clinic, Temple, Texas, and *Department of Anesthesiology, University of Miami School of Medicine, Miami, Florida*

References and Notes

1. H. Sidney, in *Life* **64**(1), 24 (5 Jan. 1968).
2. Total lung volume corresponds to the term *total lung capacity* as used in standard pulmonary physiology texts. The term *volume* is used in this report to maintain consistency in the description of gas and blood volume changes.
3. H. Rahn, in *Physiology of Breathhold Diving and the Ama of Japan*, H. Rahn, Ed. (NAS-NRC, Washington, D.C., 1965), p. 113.
4. A. Craig, *Physiologist* **10**, 149 (1967).
5. K. E. Schaefer and C. R. Carey, *Science* **131**, 1051 (1962).
6. R. D. Allison, E. L. Holmes, J. Nyboer, *J. Appl. Physiol.* **19**, 166 (1964).
7. R. D. Allison, *Physiologist* **10**, 108 (1967); *Proc. 19th Ann. Conf. Eng. Med. Biol.* **8**, 53 (1966).
8. K. E. Schaefer, C. R. Carey, J. H. Dougherty, Jr., *Fed. Proc.* **25**, 201 (1966).
9. A. B. Craig, Jr., and M. L. Medd, *J. Appl. Physiol.* **24**, 190 (1968).
10. T. Yokoyama and S. Iwasaki, in *Physiology of Breathhold Diving and the Ama of Japan*, H. Rahn, Ed. (NAS-NRC, Washington, D.C., 1965), p. 349.
11. R. G. Glassford, G. H. Y. Gaycroft, A. W. Sedgwick, R. B. J. Macnab, *J. Appl. Physiol.* **20**, 509 (1965).
12. G. F. Bond and K. E. Schaefer, in *Man's Dependence on the Earthly Atmosphere*, K. E. Schaefer, Ed. (Macmillan, New York, 1962), p. 335.
13. H. D. Bazell, S. Thurlow, C. Crowell, W. Steward, *Am. J. Physiol.* **70**, 287 (1924).
14. O. H. Gauer and J. P. Henry, *Physiol. Rev.* **43**, 423 (1963).
15. S. E. Bradley and R. J. Bing, *J. Cellular Comp. Physiol.* **19**, 229 (1942); H. V. Murdaugh, Jr., B. Schmidt-Nielsen, J. W. Wood, W. L. Mitchell, *ibid.* **58**, 261 (1961).
16. H. V. Murdaugh, Jr., W. L. Mitchell, J. C. Seaburv, H. O. Sieker, *Proc. Soc. Exptl. Biol. Med.* **108**, 16 (1961).
17. R. C. Kory, R. Callahan, H. G. Boren, J. C. Snyder, *Am. J. Med.* **30**, 243 (1961).
18. J. H. Comroe, Jr., R. E. Forster, II, A. B. DuBois, W. A. Brisco, E. Carlsen, *The Lung—Clinical Physiology and Pulmonary Function Tests* (Yearbook, Chicago, 1962), p. 325.
19. A. B. Craig, *Respiration Physiology* **5**, 14 (1968).
20. This work was made possible through the outstanding cooperation and performance of R. A. Croft, EN1 (SS)(DV) USN, who volunteered his services prior to and during his world-record breathhold dive. S. Manning, HMCS, (SS)(DV) USN, also contributed greatly to the success of the experiment. It is a pleasure to acknowledge the cooperation of Dr. Eugene Nagel, University of Miami, who provided support with equipment and encouraged staff members of the department of anesthesiology, University of Miami, to participate in the project. Supported in part by NIH grant 5-TO1-GM01714-02.

21 June 1968